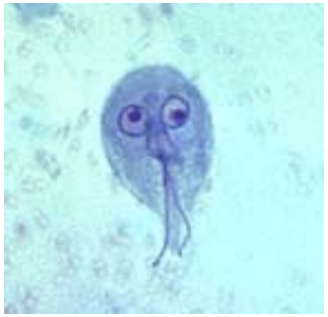
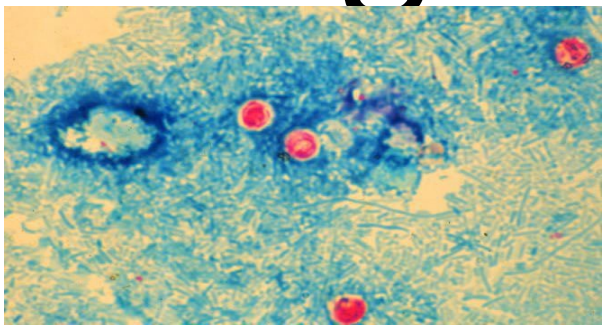
***Enterobius vermicularis* ova (pinworm)**



***Giardia lamblia* trophs**

Parasitic Lab Testing



***Cryptosporidium* oocysts**

Recap of last lecture:

- Define
  - Symbiosis:
  - Mutualism:
  - Commensalism:
  - Parasitism:
- Endo vs Ectoparasite
- Definitive vs intermediate host
- Vector

Specimen Source	Collection Procedure Used	Parasites	Laboratory Examination
Duodenum	Entero-Test	*Giardia lamblia* *Cystoisospora* spp., *Clonorchis sinensis*, *Strongyloides stercoralis*, *Fasciola hepatica*, *Encephalitozoon intestinalis*, *Enterocytozoon bieneusi*	Wet mount and permanent stains made from “washed” string
Cornea	Scrapings sent to the laboratory in airtight container	*Acanthamoeba* spp., *Naegleria* spp., *Loa loa*, *Microsporidia spp*, *Toxocara spp*, *& Toxoplasma gondii*	Wet mount and permanent stains
CSF + Body Fluids	Collect according to established procedure for each fluid type	*Naegleria fowler*, *Acanthamoeba spp*, *Toxoplasma gondii*, *Taenia solium*, *Trypanosoma spp*, *Microsporidia spp.*	Wet mount and permanent stains
Liver abscess	Aspiration	*Entamoeba histolytica*, *Fasciola hepatica*, *Leishmania Donovan*, *Echinococcus* spp., Microsporidia	Wet mount and permanent stains, culture techniques
Urine	Clean-catch urine sample	*Schistosoma hematobium*, *Trichomonas vaginalis*, Microsporidia	Examine sediment microscopically

Specimen Source	Collection Procedure Used	Parasites	Laboratory Examination
Lymph node	Surgical biopsy	*Leishmania donovani*	Impression smears
Skin	Surgical biopsy Skin snips	*Acanthamoeba* spp., *Ancylostoma* spp, *Entamoeba histolytica*, *Leishmania* spp., Microfilariae - *Onchocerca volvulus*	Permanent stains Wet mount
Mouth	Scrapings at the gum line	*Entamoeba gingivalis*	Wet mount and permanent stains
Nose	Discharge	*Naegleria fowleri* *Trachipleistophora hominis*	Wet mount and permanent stains
Genital secretions (prostate, urethra, vaginal)	Swabs submitted in sterile saline	*Trichomonas vaginalis*, Microsporidia	Wet mount of fresh specimen
Sigmoidoscopy	Aspirates, scraping, or biopsy	*Cystoisospora* spp. , Cyclospora spp., Cryptosporidium spp, Entamoeba histolytica, S. mansoni, Microsporidium spp.	Wet mount and permanent stains, histology
Sputum	Early-morning deep cough	*Paragonimus* sp,Strongyloides sp, *Ascaris* sp, *Entamoeba gingivalis*, hookworm,Microsporidia spp, & Cryptsporidium spp.	Wet mount and permanent stains Similar mounts following concentration with NaOH or *N*-acetylcysteine for mucoid specimens

Preservative	Advantages	Disadvantages
10% formalin	1. All-purpose fixative 2. Easy to prepare 3. Long shelf life 4. Good preservation of morphology of helminth eggs, larvae, protozoan cysts, coccidia, and microsporidia 5. Suitable for concentration procedures 6. Suitable for acid-fast, safranin, and chromotrope stains 7. Compatible with immunoassay kits for *Giardia lamblia* and *Cryptosporidium* spp.	1. Not suitable for some permanent stained smears such as trichrome 2. Inadequate preservation of morphology of protozoan trophozoites 3. Can interfere with polymerase chain reaction (PCR), especially after extended fixation time 4. Cannot be used with immunoassay kits for *Entamoeba histolytica/dispar* or *Entamoeba histolytica*
MIF (merthiolateiodine-formaldehyde)	1. Components both fix and stain organisms 2. Easy to prepare 3. Long shelf life 4. Useful for field surveys 5. Suitable for concentration procedures	1. Not suitable for some permanent stained smears such as trichrome 2. Inadequate preservation of the morphology of protozoan trophozoites 3. Iodine interferes with other stains and fluorescence 4. Iodine may cause distortion of protozoa
LV-PVA (polyvinyl alcohol)	1. Good preservation of morphology of protozoan trophozoites and cysts 2. Easy preparation of permanent stained smears such as trichrome (solution both preserves organisms and makes them adhere to slides) 3. Preserved samples remain stable for several months	1. Contains mercuric chloride when added to Schaudinn's solution 2. Difficult to prepare in the laboratory 3. Less suitable for concentration procedures 4. Cannot be used with immunoassay kits 5. Not suitable for acid-fast, safranin, and chromotrope stains

Preservative	Advantages	Disadvantages
SAF (sodium acetate-acetic acid-formalin)	1. Suitable for both concentration procedures and preparation of permanent stained smears 2. Easy to prepare 3. Long shelf life 4. Suitable for acid-fast, safranin, and chromotrope stains 5. Compatible with immunoassay kits for *Giardia lamblia* and *Cryptosporidium* spp.	1. Requires albumin/glycerin-coated slides for adhesion of specimens to slides 2. Permanent stains not as good as with PVA in Schaudinn's solution 3. Iron hematoxylin stain yields better results for protozoa 4. Cannot be used with immunoassay kits for *Entamoeba histolytica/dispar* or *Entamoeba histolytica*
Schaudinn's solution (mercury-based)	1. Good preservation of morphology of protozoan trophozoites and cysts 2. Easy preparation of permanent stained smears	1. Less suitable for concentration procedures 2. Contains mercuric chloride
Modified PVA (copper or zinc)	1. Permanent smears can be made and stained with trichrome 2. Zinc is preferred over copper 3. No mercuric chloride	1. Staining not consistent 2. Organism morphology may be poor 3. Copper—morphology of cysts and trophozoites is poor 4. Zinc—better morphology but not comparable to PVA 5. Careful attention must be paid to adding the correct amount of specimen to each vial with rapid fixation and thorough mixing in order to gain good staining morphology
One-vial fixatives (e.g., Ecofix, Parasafe, Proto-fix, Total-Fix, Unifix, and others that may be available)	1. Concentrate and permanent smear can be made out of one vial 2. Immunoassays can be done on most 3. No mercuric chloride	1. Certain one-vial fixatives must use certain stains 2. Color difference of stain 3. Staining not always consistent 4. Sometimes more expensive than formalin and LV-PVA

Standard Precautions - OSHA

- Gloves and gowns should be worn when handling feces
- Hands should be washed before and after handling samples (and removing gloves)
- Avoid touching hands, eyes, or mouth areas
- Clean the work environment with alcohol or 50% bleach solutions (1:2 dilution)
- Aerosolized samples after centrifugation accident should result in lab evacuation for minimum of 1 hour

Types of Specimens for Parasitology Examination

- Specimens for **Intestinal** Parasites
  1. Stool
  2. Duodenal Aspirates
  3. Sigmoidoscopy Specimen
- Urine, Vaginal or Urethral Specimens
- Blood Smears
- Tissues / Biopsy Specimen
- Sputum
- Cerebrospinal Fluid
- Serum for enzyme Immunoassay (EIA) and fluorescent antibody techniques (IFA – indirect fluoresc Ab)

Signs and Symptoms of Intestinal Parasites

- Abdominal pain
- N/V/D
- Gas/bloating
- Dysentery
- Rash or itching around the rectum
- Stomach pain
- Malaise
- Weight loss
- Possible worms in stool

General Considerations for Routine Stool Examination

- Naturally passed stools are preferred
- Collection: **Collect in dry, clean, leak proof container**
- **Avoid contamination** with urine, water, soil or any material
- Label: All specimen containers must be labeled correctly and completely
  - **Label must be on the side** not on the lid of container.
  - Patient Name, Hosp. #, Physician, Date, and Time of Collection
- All specimens placed in biohazard bag when transported to the lab
- Specimen Number: For routine parasitic exam, it is recommended that stool be submitted from:
  - **3 normal bowel movements where one sample is collected every other day or within a 10-day period**

General Considerations: Preservatives

- If there is a delay, specimen must be **preserved** since **trophozoites do not survive long**
- Stool must be preserved in the ratio of one part stool to three parts preservative (Proto-Fix)
  - Previously used preservatives:
    - 10 % **Formalin** for concentration method
    - **PVA** (polyvinyl alcohol) for permanent stain
    - **SAF**
  - Current preservative:
    - **Proto-Fix clear** (formalin/ ethanol /methanol/ ethyl acetate) – one preservative for concentration and permanent stain
  - Bloody, watery and slimy areas **must** be preserved.
  - **Sample must be taken from the outer edge, middle and ends of the formed stool**

General Considerations: Timing

- Processing Fresh Specimens: place in Proto-Fix
  - **Liquid stool- within 30 minutes** of passage (not within 30 minutes of arrival to the lab)
  - Semisolid or **soft stool** - **within an hour**
  - **Formed stool** - **within 24 hrs**., may be refrigerated for 1 to 2 days if exam is delayed (do not guarantee recovery of all parasites)
    - Hookworm ova may mature and hatch if allowed to remain at RT and may be confused with Strongyloides larva
- **Store in refrigerator at 4 C**
- Storage at room temp 25 C or in incubator 37 C will increase rate of disintegration and enhance growth of bacteria

General considerations: Precautions

- **Unacceptable substances** in stool sample: kaolin, antacids, mineral oil, antidiarrheal preparations, laxatives, barium or bismuth
- **Time of collection**: prior to administration of antimicrobials or two weeks after the antibiotic therapy has ended
- Precautions should be taken even with preserved stool specimens.
  - **Formalin takes days or weeks to kill cysts or oocysts**
    - Ascaris lumbricoides ova may continue to develop and are infectious even when preserved in formalin
    - MLS may be infected if not using aseptic technique

General considerations: Precautions

- **Universal precautions** and standard microbiological practices must be observed when handling specimens:
  - **Wear protective equipment** - gloves, lab coats
  - **Use biological safety cabinet**
  - Do not eat, drink, apply cosmetics or manipulate contact lenses in the work area
  - **Decontaminate the work area**
  - **Hand washing**

Macroscopic Examination

- Note the **consistency** of the specimen
  - Consistency – trophozoites vs cysts, and eggs + larvae
  - Exterior stool surface - tapeworms
  - Interior stool contents - nematodes
  - Presence of blood, bile, mucus – GI bleed and trophozoites
- **Break up stool** with applicator stick for presence of adult worm within
  - Ascaris
- Examine for **mucus or blood**
- **Lower** Bristol Stool score = **more cysts**
- **Higher** Bristol Stool score = **more trophozoites**

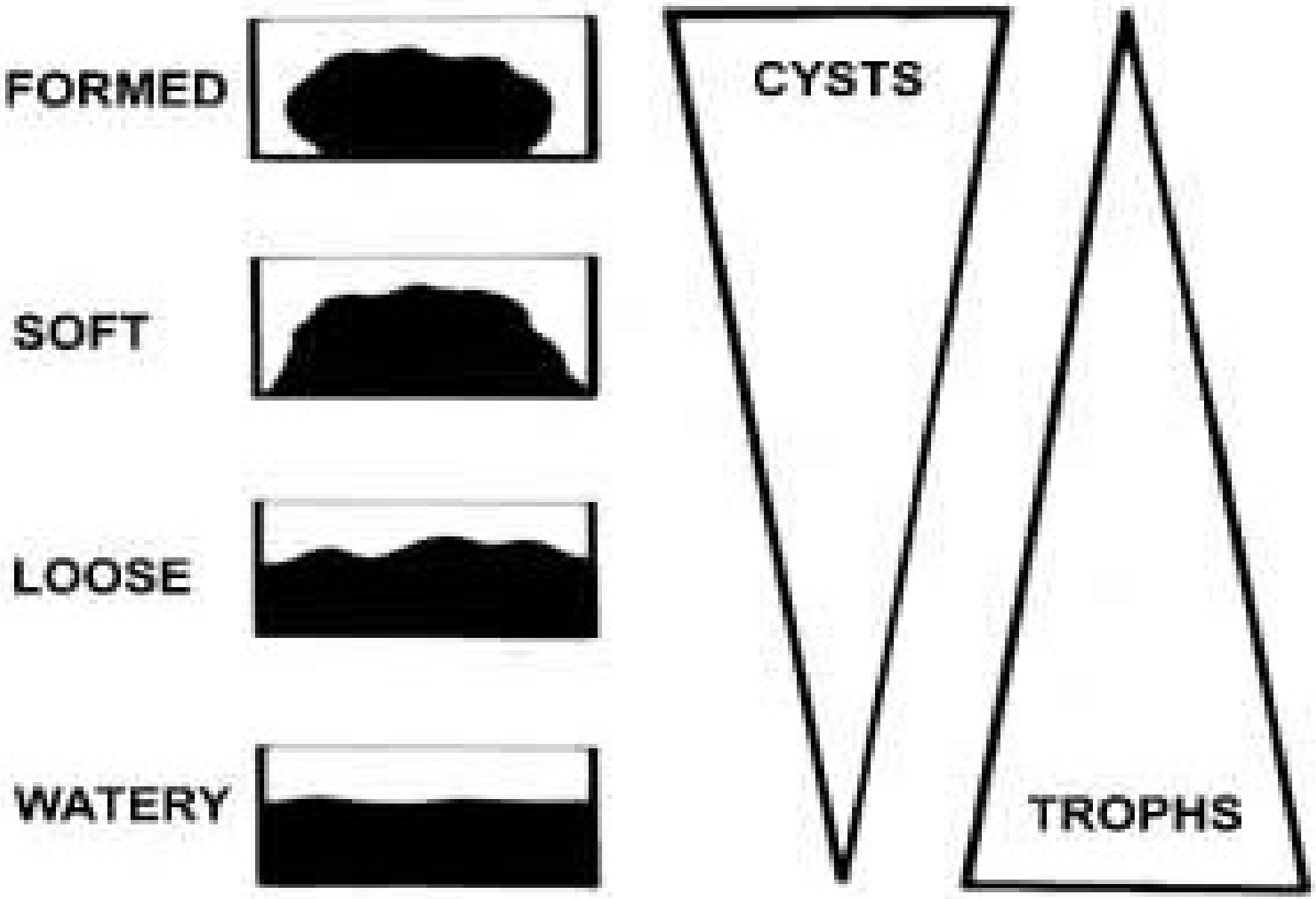
THE BRISTOL STOOL FORM SCALE (for children)

choose your POO!

type 1		looks like: **rabbit droppings** Separate hard lumps, like nuts (hard to pass)
type 2		looks like: **bunch of grapes** Sausage-shaped but lumpy
type 3		looks like: **corn on cob** Like a sausage but with cracks on its surface
type 4		looks like: **sausage** Like a sausage or snake, smooth and soft
type 5		looks like: **chicken nuggets** Soft blobs with clear-cut edges (passed easily)
type 6		looks like: **porridge** Fluffy pieces with ragged edges, a mushy stool
type 7		looks like: **gravy** Watery, no solid pieces ENTIRELY LIQUID

Macroscopic Evaluation

- If adult worms are found:
  - Examine and identify
- Tapeworms are identified by looking at scolex and proglottids
  - T. solium eggs are highly infectious so be careful when handling
- Evaluate proglottid using 10% formalin container, adding proglottid into glycerin or lactophenol
  - May inject India prep ink with syringe to enhance findings
  - Flatten tapeworm between two glass slides and evaluate
- Artifacts associated with delusional parasitosis include:
  - Insects, threads, fibers, plant materials


FORMED
SOFT
LOOSE
WATERY
CYSTS
TROPHS

Microscopic Evaluation

- 3 separate procedures for ID of parasites:
- Direct saline or iodine wet mount
  - Rapid screen for trophozoites (motility) in liquid stool
- Fecal concentration technique
  - Increases likelihood of recovering eggs, larvae, and cysts. Trophozoites may be destroyed
- Permanent staining technique
  - Identifying protozoans, cysts, and eggs after concentration

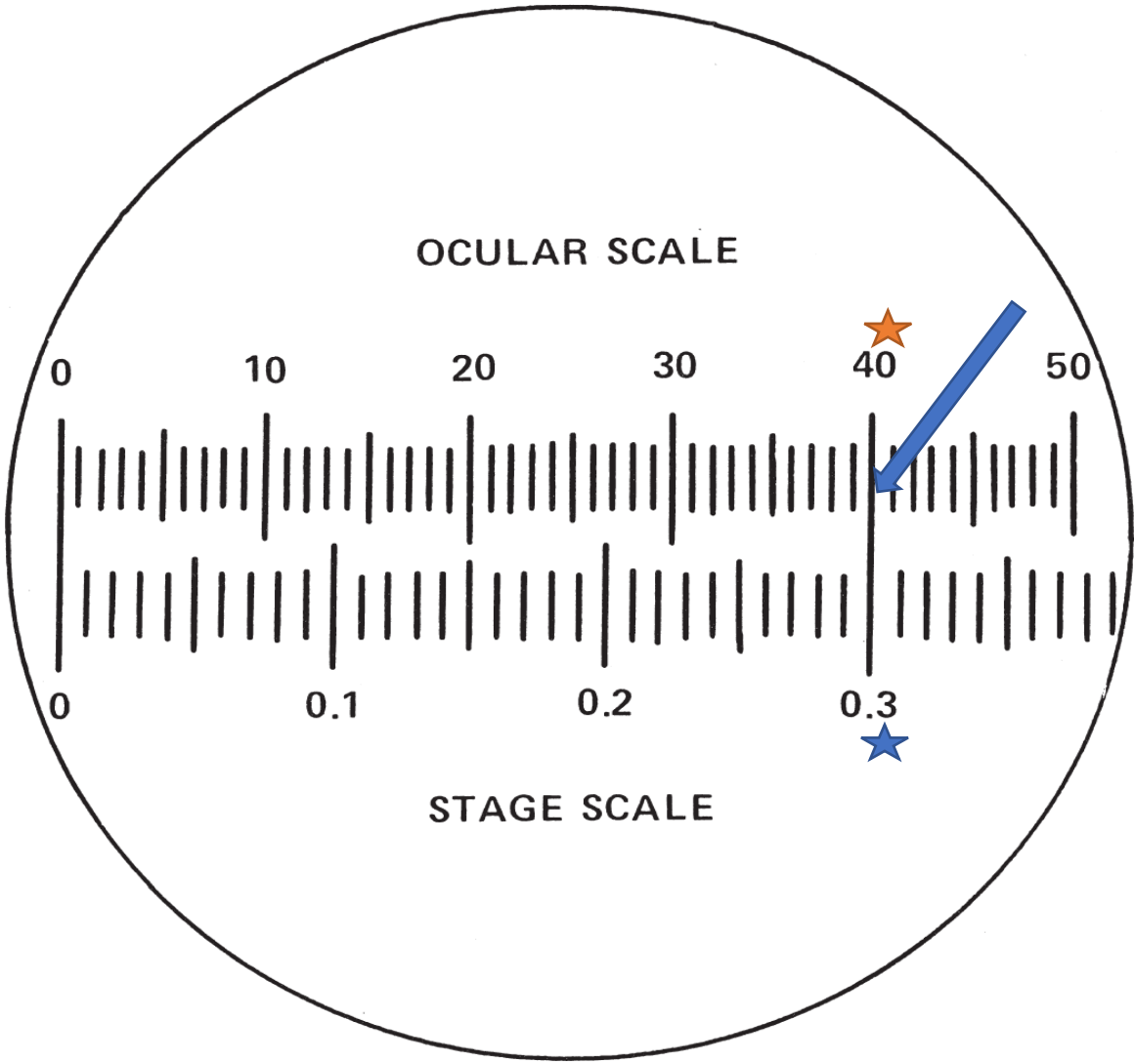
Ocular Micrometer Calibration

- Whenever a microscopic examination is performed, a calibrated ocular micrometer should be used because size can determine true parasites from artifacts and debris
- Procedure:
  - Install the ocular scale eye piece
  - Place a stage scale micrometer on the stage and focus under 10X
    - Stage scale = 1mm and calibrated in 0.01 mm increments (10µm)
  - Line up ocular scale with stage scale at left edge
  - Find an increment where the two scales are superimposed
  - Use equation* and write down calibration
  - Repeat for 40X and 100X
- Equation* → $\frac{\text{(Number of stage micrometer spaces} \times 10\ \mu\text{m)}}{\text{(Number of ocular micrometer spaces)}}$

  = µm/ocular space

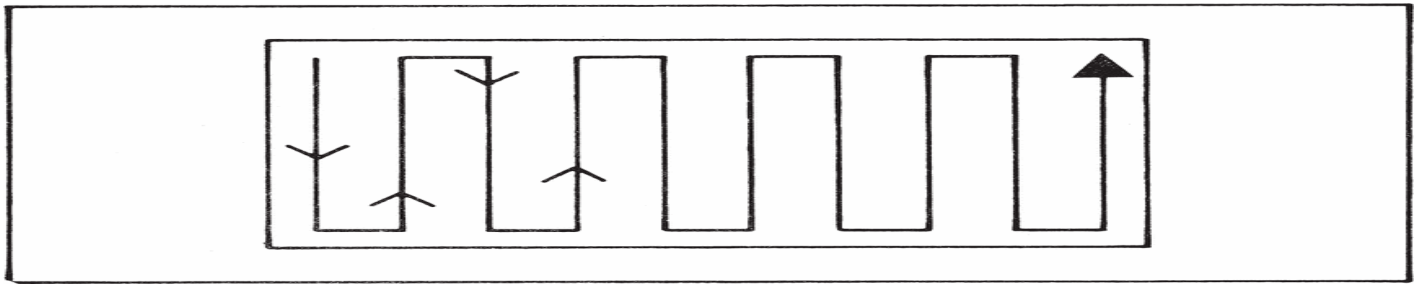
Example

- For example, note that the **40th ocular scale** line is exactly superimposed over the **30th stage scale** line (0.3 mm)
- Use data in the formula: (30 × 10)/40 = 7.5 µm per ocular space



Direct Wet Mount

- Testing on fresh, liquid stool by using applicator stick to mix feces with a drop of saline on a slide (not too thick a sample)
- Add a coverslip
- Move in a battlement pattern under 10X (lpf) looking for eggs, larvae, or protozoa
- Switch to 40X (high dry) to screen for protozoa and eggs in battlement pattern
- Common source of error → too much light which reduces contrast with background
  - Iodine may be added to enhance staining
  - Repeat procedure but with iodine and a coverslip (may be done on same slide)

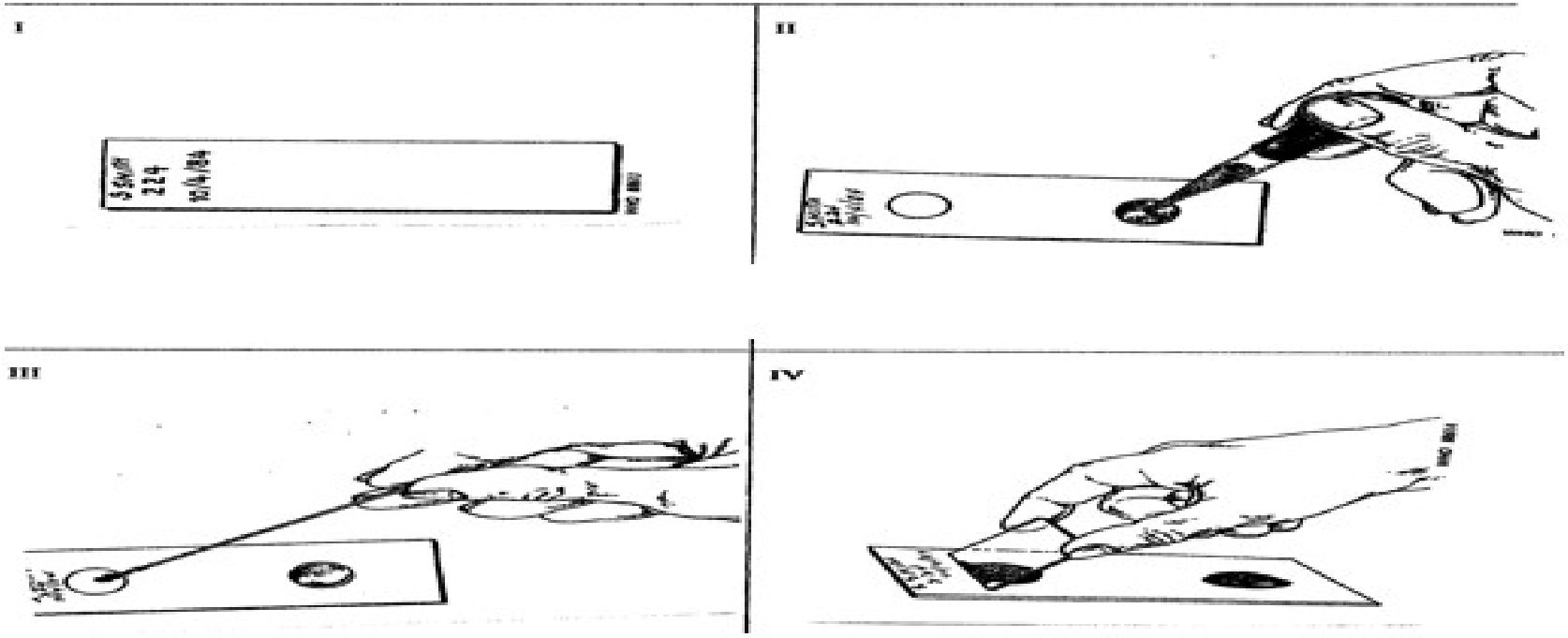


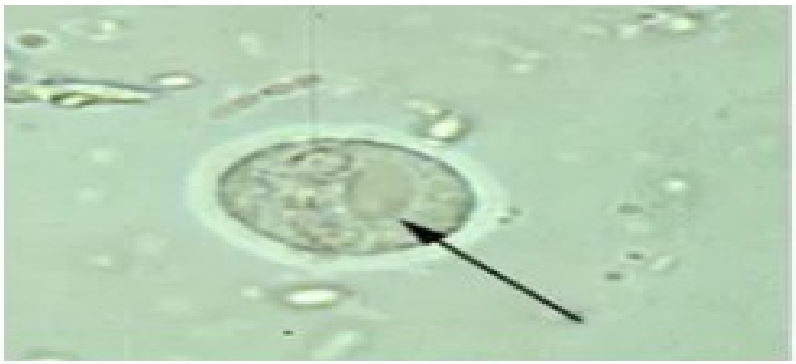
Direct Wet Mount

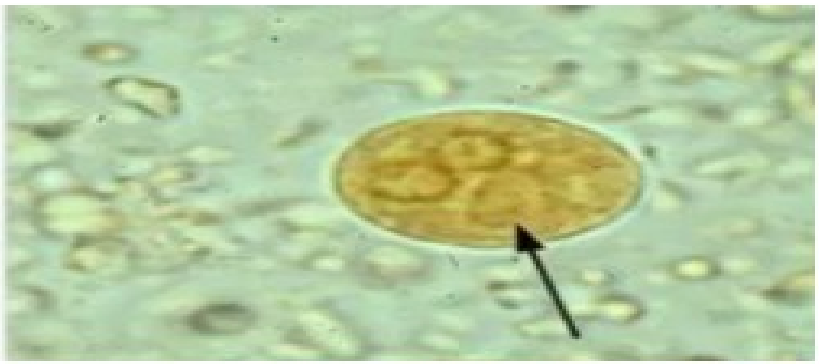
- If parasites are seen:
  - Identify and report out based on scientific name
  - Report out stage of parasite seen (egg, trophozoite, larvae, cyst, etc.)
  - Eosinophils (Charcot-Leyden Crystals), RBCs, Yeast, WBCs may be semi-quantitively reported
- Wet Mount QC:
  - Performed weekly for iodine solution
    - Should be clear but dark brown in color
  - Positive control → adding human buffy coat to negative stool sample
    - WBC cytoplasm should be yellow-gold color and similar to protozoan trophozoite color
    - A well-preserved previous positive may also be used
- Ocular micrometer should be calibrated annually

Direct Wet Mounts

Saline Wet Mount vs Iodine Wet Mounts







Concentration Techniques

- Requires a fresh sample preserved in formalin from SAF or proto-fix collection
- Purpose of concentration method
  - **Concentrate** parasites in low number
  - Small volume of fluid specimen
  - **Remove** fecal debris
  - Protozoan cyst, helminth eggs and larvae are **detected**
  - Protozoan trophozoites do **not** survive (except with Proto-Fix)
  - May be examine separately with and without iodine stain
- Sedimentation and Flotation Method
  - Both methods are based on difference in specific gravity between parasite and the concentrating solutions

Sedimentation Technique

- Organisms are concentrated in the sediment at the bottom of centrifuge tube
- **Concentrates a greater diversity of parasites than flotation method**
- Use **solutions** with **lower specific gravity than the parasites**
  - Creates layers using surfactant, formalin, and ethyl acetate

1. **Formalin-Ethyl Acetate** Method
   - replaced Formalin-Ether due to hazard associated with ether
2. **Proto-Fix:** Formalin/ethanol/methanol/ethyl acetate
   - Single vial, no PVA, no heavy metals, reduced formalin content; transport & preservation
   - Use for wet preps, concentration, and permanent stain

Flotation Technique

- **Use liquids with a higher specific gravity than that of the eggs or cysts**
- The concentrating solution must have a final specific gravity of **1.18** (1.2 can be used for formalin-preserved stool)
  - Zinc Sulfate method
    - Zinc sulfate causes operculated eggs to open or collapse
    - Tends to distort protozoan cysts
    - Heavy operculated eggs and infertile Ascaris eggs do not float

Diphyllobothrium latum egg

Flotation Technique

- Most organisms tend to settle after 30 mins so timing is crucial
  - **Sheathe Sugar Method-**
    - For detection of Cryptosporidium sp
    - Better visibility of oocysts due to better refractility
    - Can be used with fresh or formalin-fixed feces

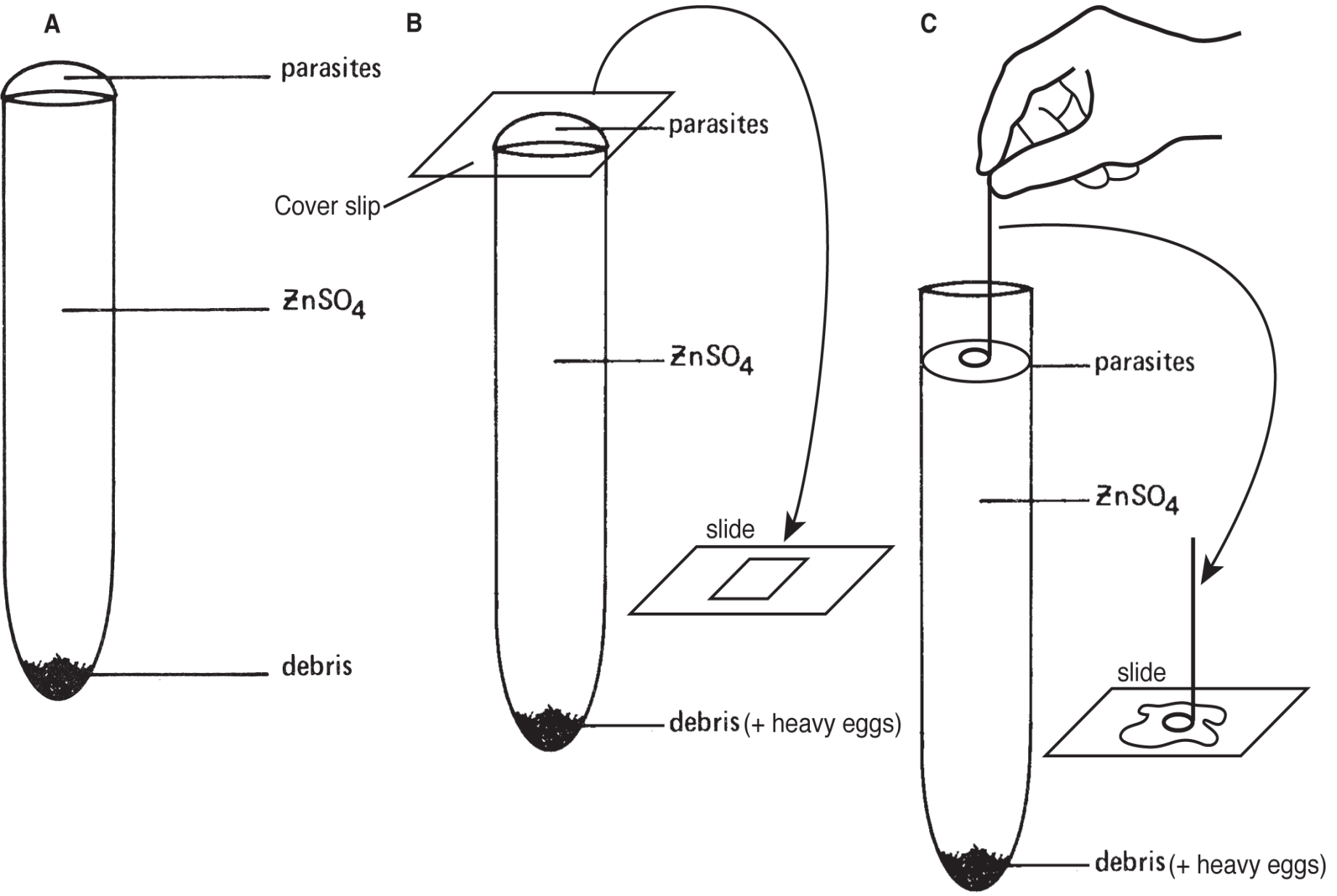
Zinc Floatation Method

- Uses liquids of higher specific gravity than eggs or cysts so eggs float above or near the surface
- Concentration solution used should be 1.18 or higher
- Common reagents include Zinc Sulfate
- Limitations:
  - Not great for recovery of Ascaris eggs
  - High specific gravity solution kills trophozoites and breaks fragile eggs like Hymenolepis nana

Zinc Floatation Method: Procedure

- Prepare sample in formalin and allow to sit for 30 minutes
- Strain the sample into a 15 mL conical vial through two pieces of gauze
- Wash twice with saline (centrifugate each time to pellet and remove supernatant)
- Resuspend pellet in 12 mL of zinc sulfate
- Centrifugate for 2 minutes at 1500 rpm and allow slow brake and then:
- A- Slowly add zinc sulfate until an inverted meniscus appears
  - Slowly put coverslip through bottom of meniscus and let meniscus settle on coverslip for 10 minutes
- B- Alternatively, use a loop to scoop the surface content onto a coverslip

Zinc Floatation Testing



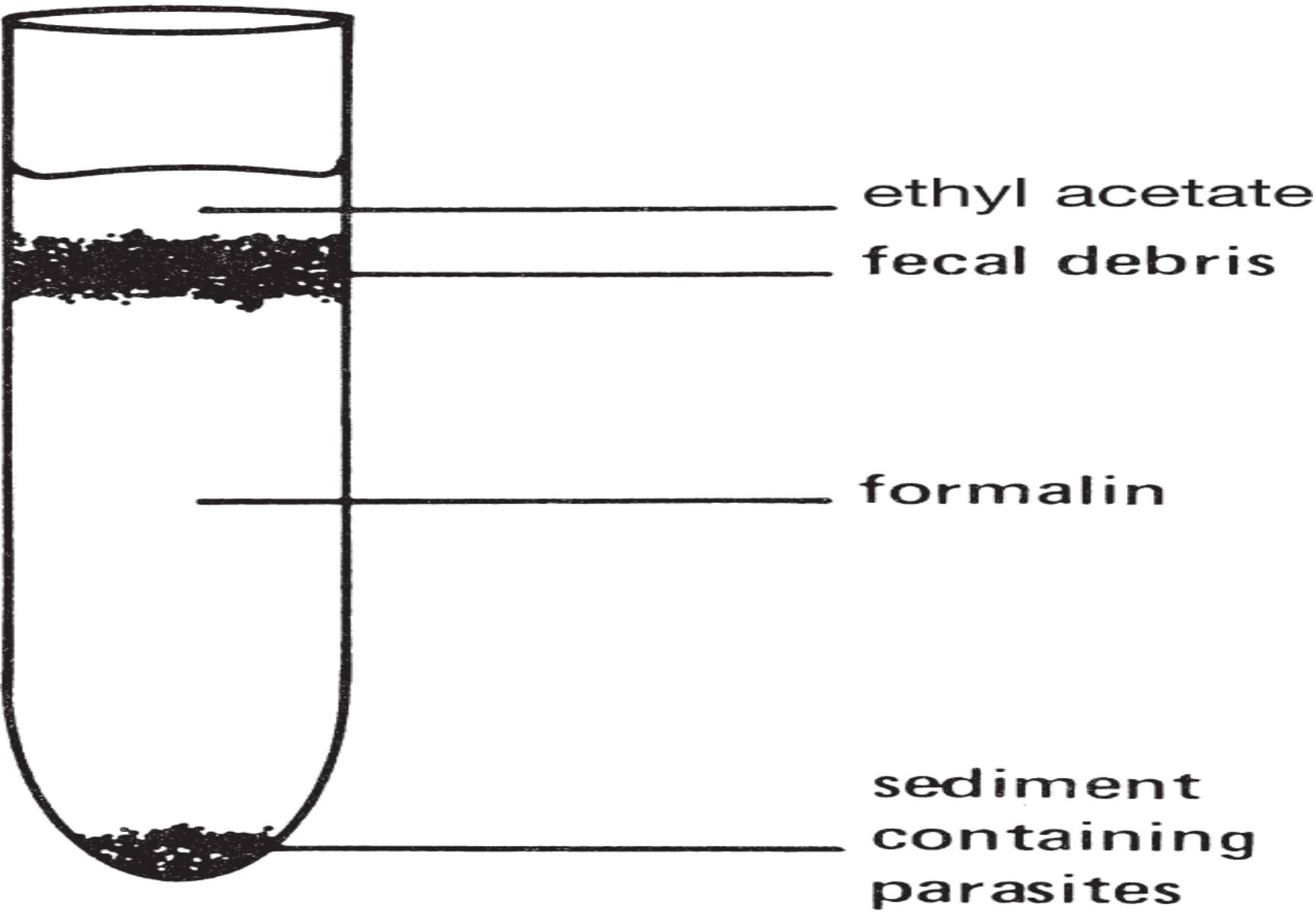
Formalin-Ethyl Acetate Method

1. Add 5 – 15 g of fresh feces to a container with 10-15 mL of 10% formalin
2. Mix and fixate for 30 minutes
3. Transfer mixture into a 15mL conical vial between 2 pieces of gauze
4. Add saline to the top of 15 mL conical vial
5. Centrifugate the suspension for 1500 rpm for 10 minutes
6. Decant supernatant into disinfectant and resuspend sediment pellet with saline
7. Add 7 mL of 10% formalin and 4 mL of ethyl acetate
8. Cap and shake tube for 30 seconds
9. Centrifugate for 10 minutes and 4 layers should be in tube
10. Remove upper debris and decant supernatant into disinfectant
11. Resuspend pellet in drop of saline and test
    - Permanent staining, wet mount staining, etc.

Formalin – Ethyl Acetate Method

- May be used with PVA but requires additional steps of washing before step 1
  - Cystoisospora belli may be missed with PVA samples
- SAF may start at step 3 on previous slide
- Centrifugation speeds are important for recovery of Cryptosporidium spp as false negatives may exist if centrifugation is too low
- If washing occurs, do not wash more than twice because you may wash away parasites (false negative)

Formalin – Ethyl Acetate Method



Concentration Technique Reporting

- Regardless of Sedimentation or Floatation test:
- If parasites are seen:
  - Identify and report out based on scientific name
  - Report out stage of parasite seen (egg, trophozoite, larvae, cyst, etc.)
  - Eosinophils (Charcot-Leyden Crystals), RBCs, Yeast, WBCs may be semi-quantitively reported (few, moderate, many)
- QC should be performed on solution to ensure it is not contaminated
- Calibration of ocular micrometer as needed (annually)

Permanent Stained Smear for Intestinal Parasites

- **Final part of the complete fecal examination**
- Permanent stained smears provide a permanent record

1.Trichrome Stain (Wheatley)

- Smears made from PVA-fixed or Proto-fix preserved specimens
- Identification and confirmation of protozoan cysts and trophozoites
  - Nuclear structures are clearly visible, red to purple in color
  - Cytoplasm stain greenish-blue or green
  - Inclusions as chromatoid bodies or RBCs stain purple
  - Background = light green
- Useful for Entamoeba spp, blasotocystis, giardia

2.Modified Acid Fast Staining (Kinyoun modified)

- Useful for Cryptosporidium, Isospora, Cyclospora
  - Oocysts appear magenta against a blue background

Trichrome Staining Procedure

1. Apply a thin layer of fresh feces on a slide and place the slide in Schaudinn's solution for 30 minutes at room temperature
2. Rinse with 70% alcohol to remove the fixative (omit if PVA was used) for 2 minutes
3. Place in two successive sequences of 70% ethanols for 5 minutes each
4. Place in Trichrome stain for 15 minutes
5. Rinse in 90% ethanol for a couple seconds
6. Place in clean Xylene for 5 minutes
7. Add Permount or some mounting media with a cover slip
8. Allow to air dry and exam under 100X for 200-300 fields before reporting negative results

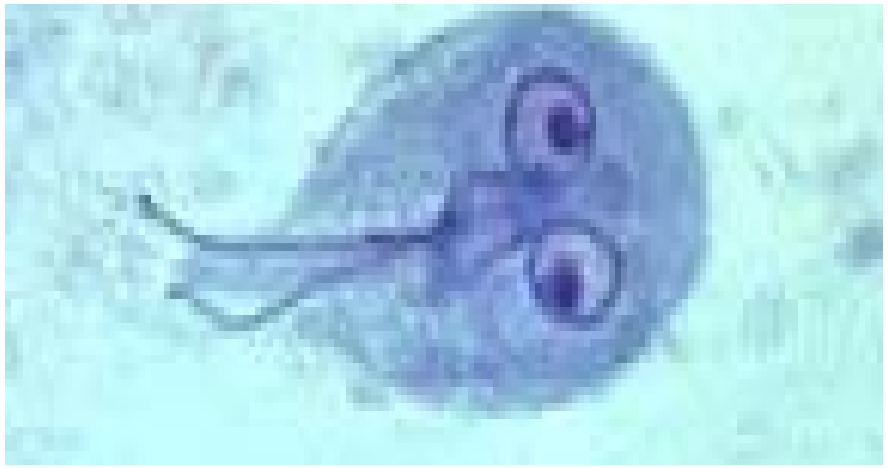
- CAP recommends that stains are replaced and checked monthly (at least)

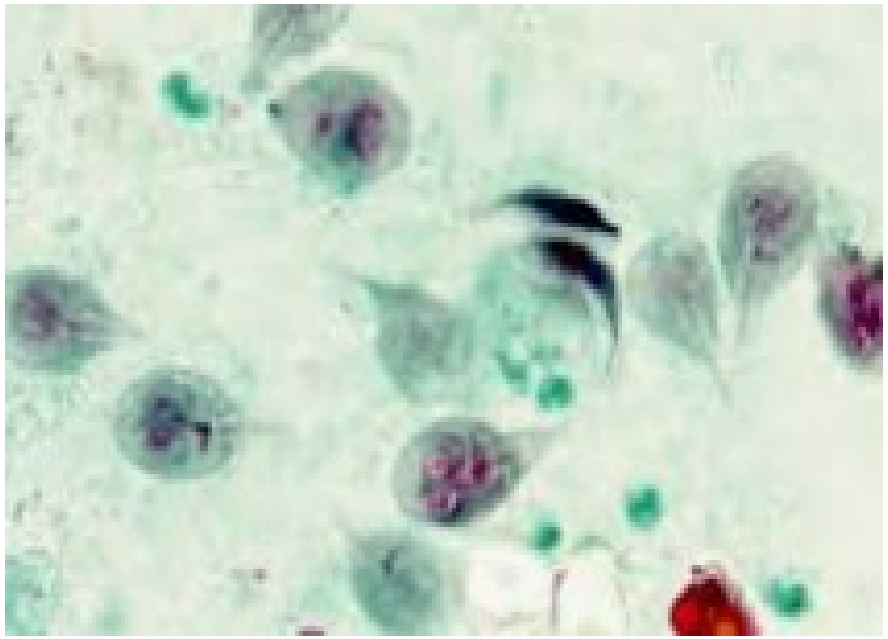
Modified Trichrome Staining

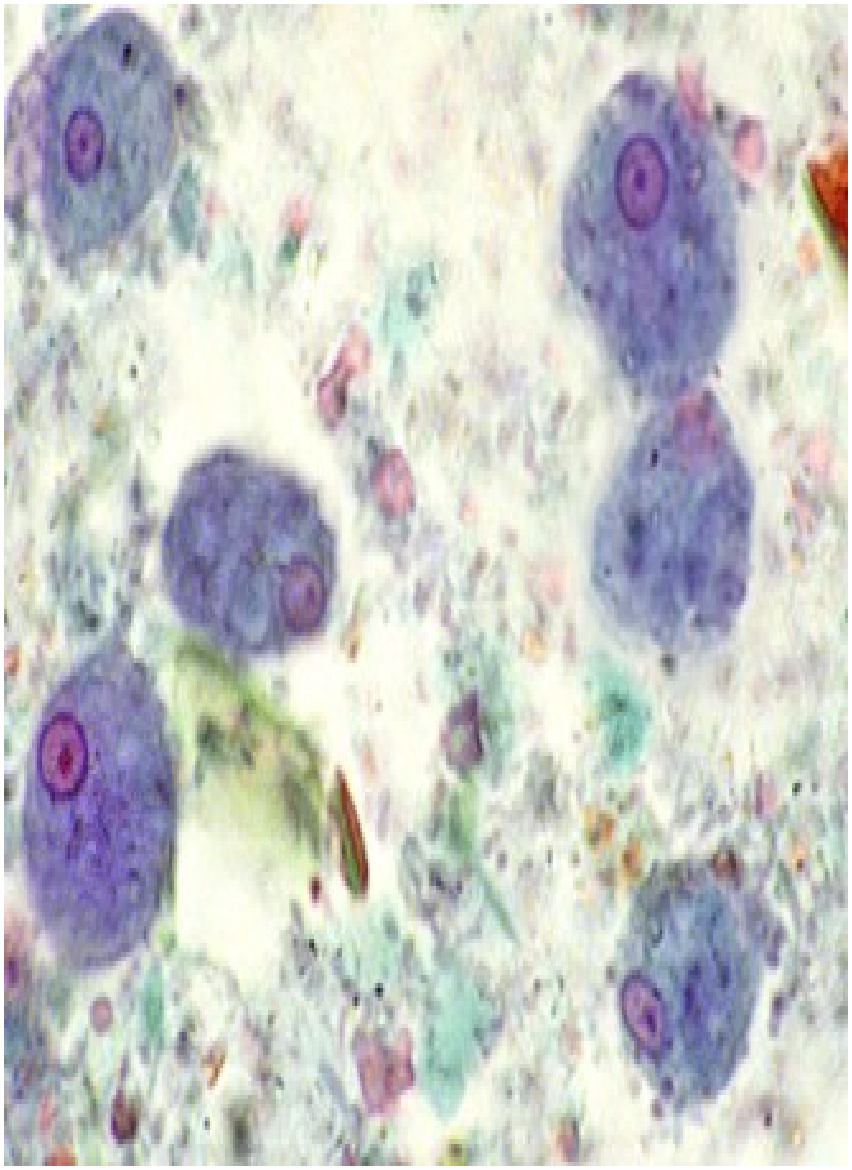
1. Add 10um of feces to a microscope slide and dry the slide
2. Soak slide in 100% methanol for 5 minutes and allow to air dry
3. Add Trichrome stain for 90 minutes
4. Since the slide in acid alcohol for up to 10 minutes
5. Rinse quickly with 95% ethanol and soak for 5 minutes in 95% ethanol
6. Place in absolute ethanol for 10 minutes
7. Place in Xylene for 10 minutes

- Using mounting solution (permount) and examine under 100X oil for 200-300 fields before reporting negative results

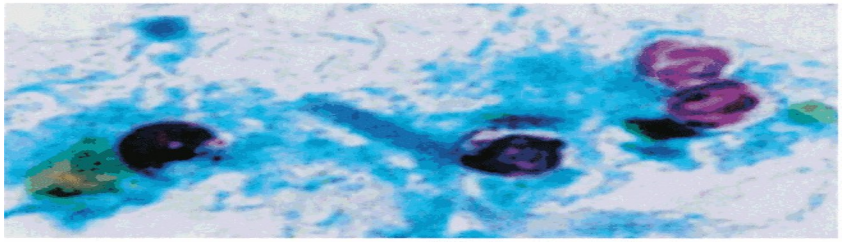
Trichrome Stain – 400X



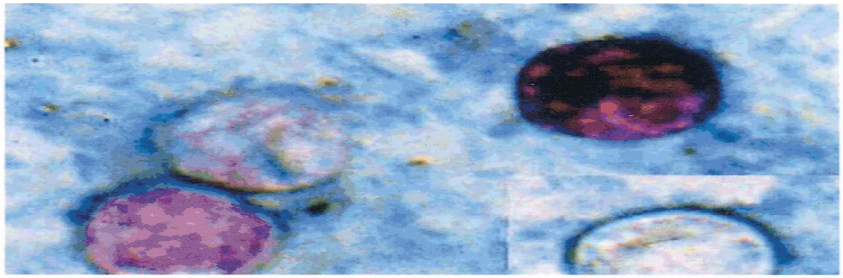




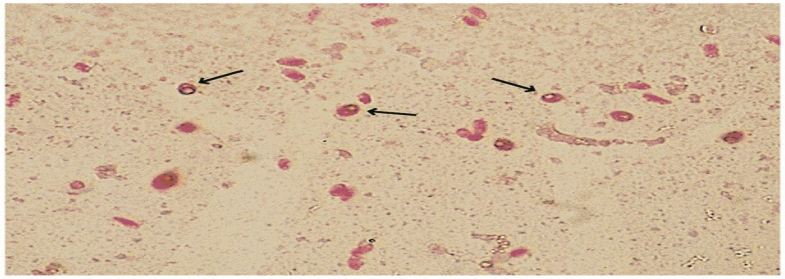
Artifacts vs Real Parasites



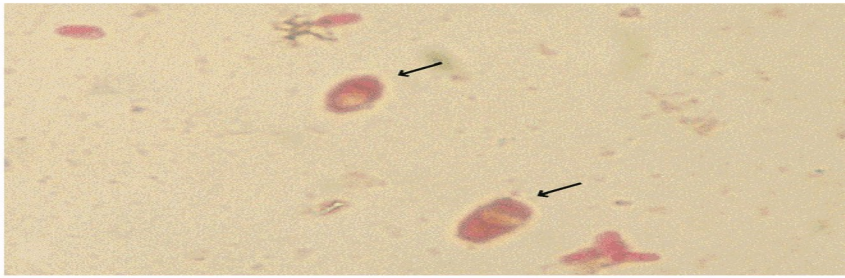
129.



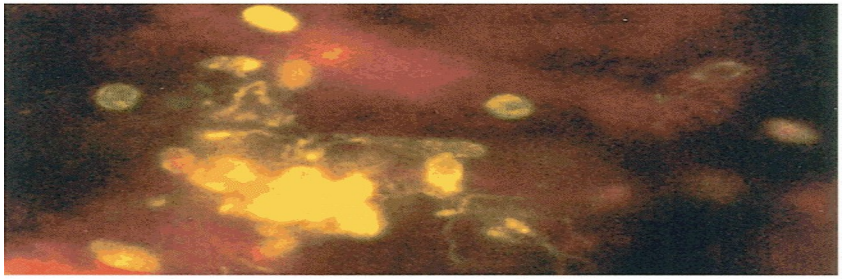
130. 10µm



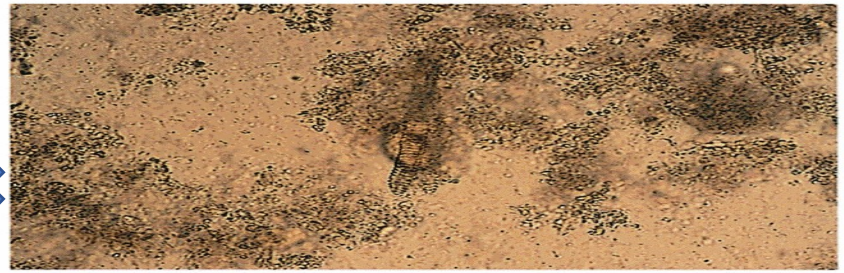
131. 10µm



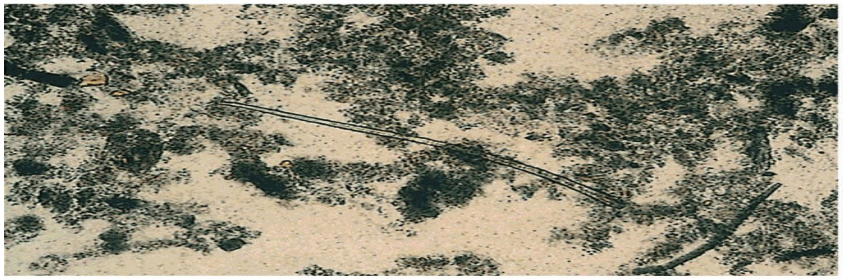
132. 5µm



133. 5µm

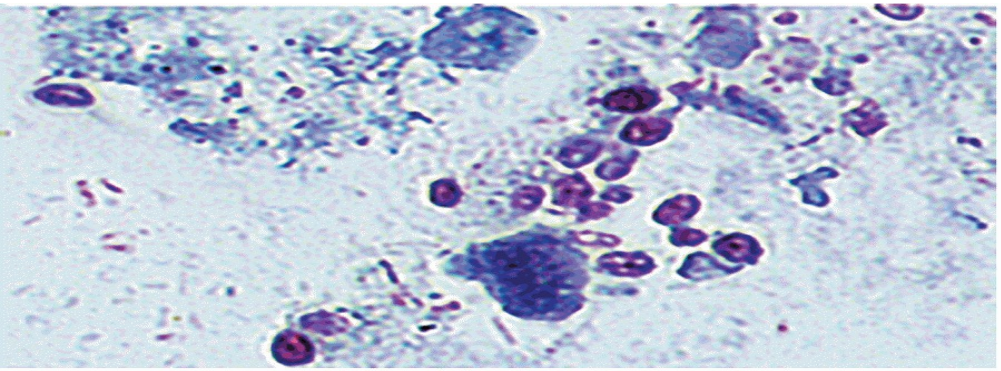


134.

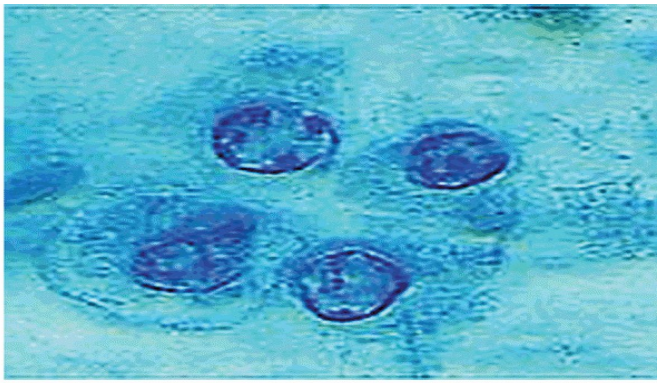


135.

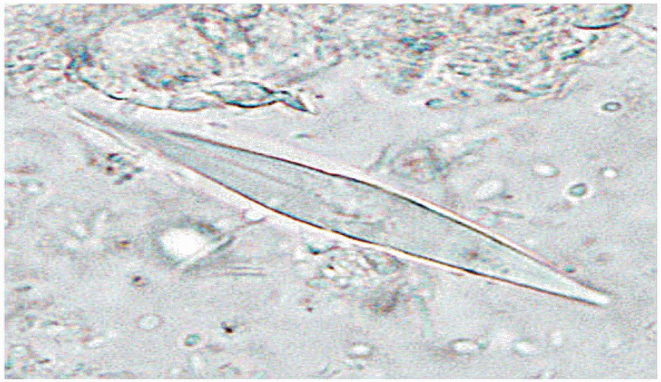
- Direct Mount Artifacts may be mistaken for parasites to the untrained eye



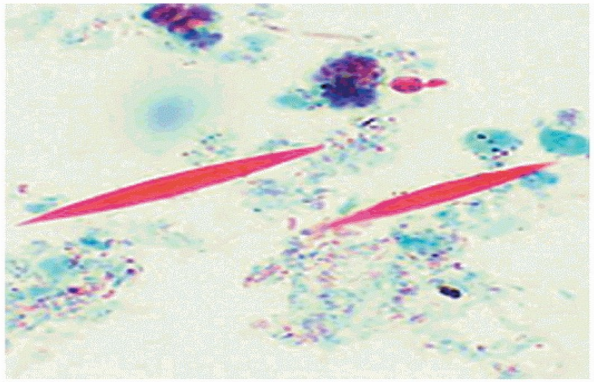
136A.



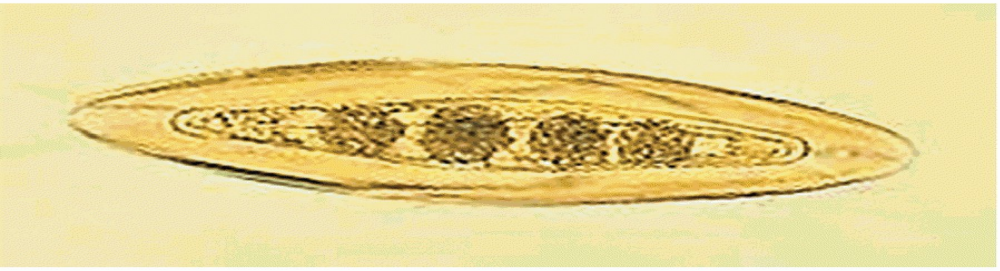
136B.



137A.



137B.



138A.

138B.

Other Specimens for Intestinal Parasites

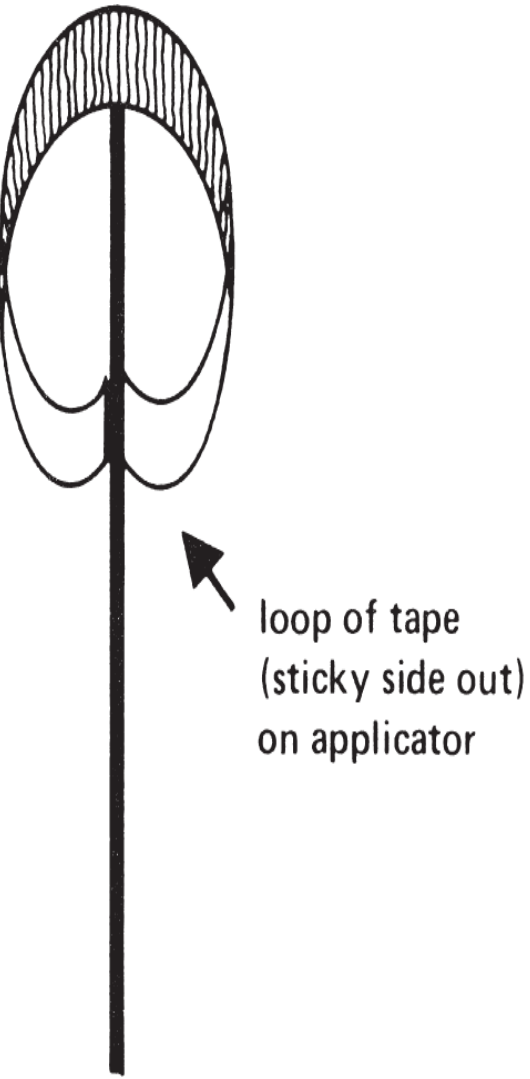
- **Cellophane Tape Preparation** for Pinworm
  - Pinworms are rarely found in stool but lay eggs at night
  - Should be collected first thing in the morning
- **Duodenal Aspirates**
  - Suspected cases of Giardiasis or Strongyloidiasis
  - Obtained by intubation
  - Examined by wet mount or preserved in PVA for permanent staining
- **Urogenital Specimen** (Urine, Vaginal or Urethral)
  - T. vaginalis, S. haematobium eggs and E. vermicularis eggs in urine
  - Trichomonas in vaginal and urethral specimen
    - wet mount and/or culture, or PCR
    - permanent stain

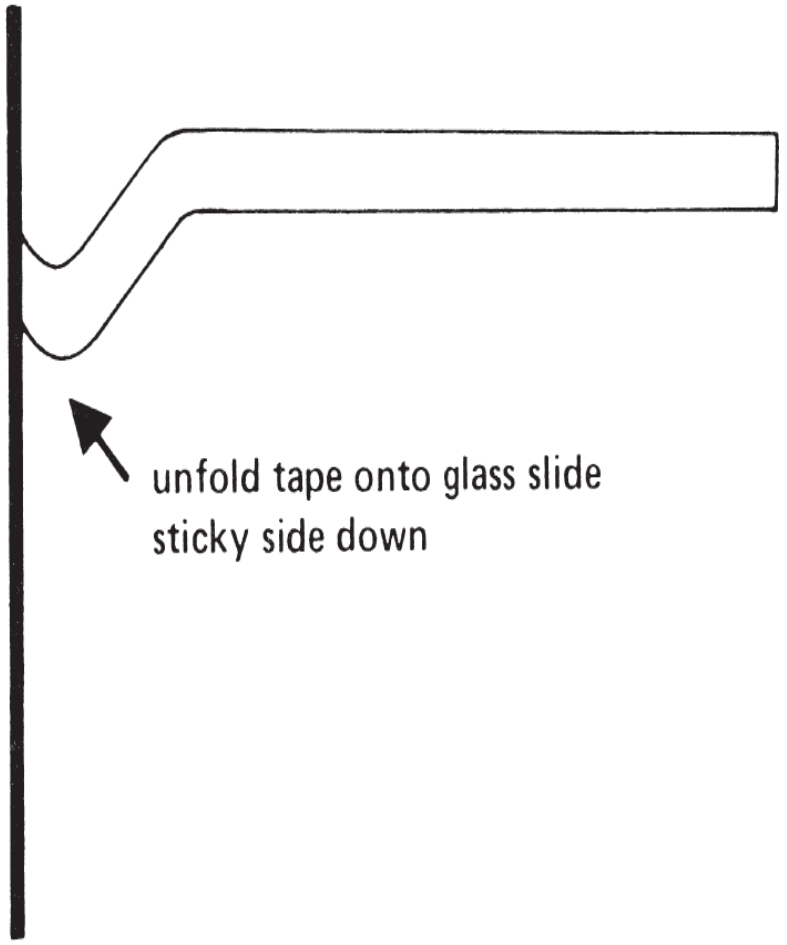
Cellophane Tape Prep for Pinworm

- Used to isolate E. vermicularis eggs as female nematode migrates to anus to deposit eggs in perianal region at night

1. Fold edges of cellophane tape around a tongue depressor or applicator stick
2. Spread the cheeks and apply the tape facing the anal area
3. Use a rocking motion to cover as much surface area as possible
4. Remove tape and apply it to a microscopic slide
5. Evaluate under 10X under low light for increased contrast as eggs are colorless

Cellophane Tape Test





Other Specimens for Parasitology Examination

- **Sigmoidoscopy Specimen**
  - Scrappings or aspirates for amoeba or cryptosporidium
  - Wet mount or permanent stained (PVA fixed)
- **Sputum**
  - Direct mount for Strongyloides larva and P. westermani (lung fluke) egg
  - Permanent stained smear for E. histolytica in cases of pulmonary abscess
    - Add 3% NaOH to emulsify sputum
- **Cerebrospinal Fluid**
  - Suspected cases of amebic meningitis and sleeping sickness
  - Motile trophozoites
  - For meningitis caused by Naegleria fowleri

Specimens for Blood and Tissue Parasites

- **Blood**
  - **Thin and thick smears for malaria and other blood parasites**
  - Wet preparation
  - buffy coat for Trypanosomas, Leishmania
- **Aspirates**
  - Liquid specimens from a variety of sites when routine exam fail as fine needle aspirates of hydatid cyst
- **Tissue and Biopsy Materials**
  - Materials obtained from lymph nodes
    - Useful for trypanosomes and leishmania as well!
  - Muscle tissue as in Trichinosis
  - Bone marrow
  - Skin ulcers
    - Cutaneous leishmaniasis

Blood Smears for Parasites

- Thick and thin smears are prepared, stained, and evaluated for Plasmodium, Trypanosomes, microfilaria spp, and Babesia spp, and Leishmania spp.
- EDTA samples are preferred from capillary blood collection from a free-flowing drop of blood (no alcohol contamination)
- Thin smears are usual for identification of the parasite but in low parasitic burdens may be hard to find
  - 200-300 fields are observed before calling negative
- Thick smears are good for quantitative detection of parasites by may distort parasite appearance

Blood Smear Preparation

- Thin smears are made similar to a peripheral blood smear in Hematology, air dried, and Giemsa stained
- Before resulting:
  - Thin smears are typically evaluated for 30 minutes under 100 X
  - Thick smears are typically evaluated for 300 fields under 100X
- Thick smears are made by using the corner of another slide to spread a drop of blood in a circular manner about the size of a dime or nickel
  - Some hospitals may lake the blood, which lyses RBCs
  - RBCs are more translucent for easier parasite detection
- Buffy coat may be taken from blood samples for patients suspected of bloodborne parasites for identifying gametocytes

Blood Smear Preparation : Giemsa Staining

- Thin smears:
  - Blood is immersed in absolute methyl alcohol and dried thoroughly
  - Stained in 1:100, 1:50, or 1:20 Giemsa for 120, 45, 20 minutes respectively
  - Rinse excess Giemsa and do not blot
  - Examine under 100X
- Thick smears:
  - Unlaked blood is stained under 1:50 Giemsa for 50 minutes
  - Allow to air dry and examine under 100X

Blood Smear Reporting

- Smears should be prepared within 1 hour after EDTA collection
- Malaria, Babesia, Trypanosomes, Leishmania cytosol stains blue and nuclear content stains red
- RBCs are pale red
- WBCs are purple
- Identify detected parasites to scientific name and speciate based on hospital SOP

Knott Technique for Concentrating Microfilariae

- Used for blood or tissue nematode infections
- 2 mL of whole blood via venipuncture and added into 10mL of 2% formalin
- Thoroughly mix by inversion
- Centrifuge the tube for 5 minutes at 1000 rpm and decant supernatant
- Add sediment to a slide
  - Examine wet for microfilariae
  - Examine dry if allowed to dry overnight and stained with Giemsa for 45 minutes following thick smear procedure

Other Diagnostic Procedures in Parasitology

- Detection of Parasite Antigen
  - Direct fluorescent antibody (**DFA**)
  - Enzyme immunoassay (**EIA**)
  - Rapid dipstick-like test
- Specimen for Antigen Detection: fresh or preserved stool samples
  - Amebiasis
    - EIA kits - use monoclonal antibodies
  - Cryptosporidiosis
    - DFA, modified Acid Fast, EIA rapid tests
    - some kits are combined for Giardia, Cryptosporidium and E. histolytica
  - Giardiasis
    - DFA, EIA, rapid tests
  - Trichomoniasis
    - DFA, EIA using enzyme labeled monoclonal antibodies, PCR

Agar Plate Culture for Strongyloides stercoralis

- Most people may be asymptomatic but in immunocompromised patients, this nematode may be rapidly fatal
- Place 2 gram of fresh stool in a nutrient agar plate
- Seal the plate with cellophane and incubate at RT for 48 hours
- Look for evidence of larvae migration
  - Positive shows larval migration away from inoculation site
- Confirm positive by washing the plate with 10% formalin and performing sedimentation technique for direct examination

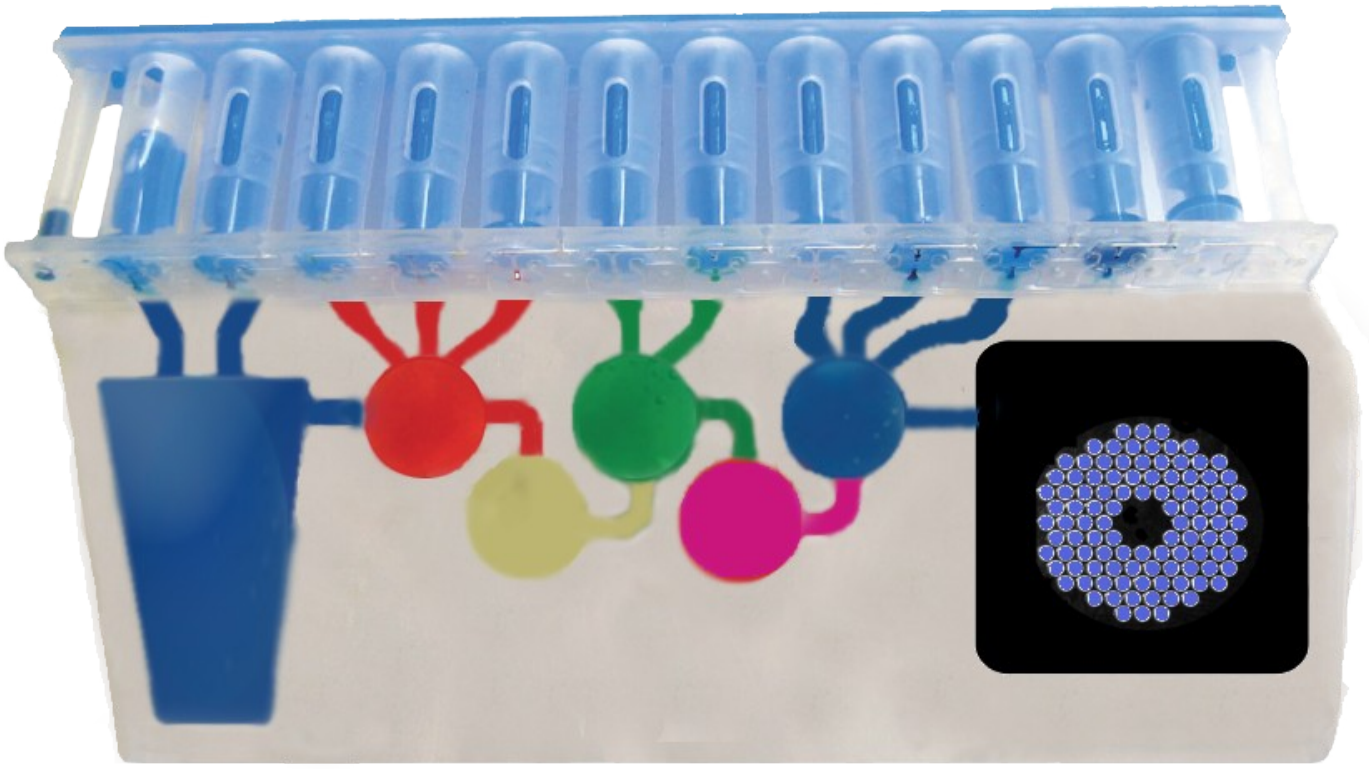
Plate Culture for Protozoans

- Routine diagnosis of protozoal infections are made without culture
- Some labs use agars that are useful for identifying Acanthamoeba spp, Entamoeba histolytica, Naegleria folweri, Toxoplasma gondii, and Trichomonas vaginalis
- Agars and broths include:
  - Boeck and Drbohlav medium, Cleveland-Collier medium, or McQuay diphasic charcoal medium) or a nutritive fluid (Balamuth medium)
  - Diamond medium is used primarily in research centers and is most useful when stock cultures of E. histolytica must be maintained
  - Leishmania spp. and Trypanosoma cruzi can be cultured using Novy-MacNeal-Nicolle (NNN) medium
- Only fresh fecal specimens (less than 6 hours old) should be used
- Positive reactions typically involves clearing of bacteria on agar plates as they eat bacteria

Other Diagnostic Procedures

- **Molecular (PCR) Diagnostic Test**
  - Specimen must be collected without preservatives
    - Either frozen or refrigerated at 40 C
  - **Stool placed into Cary-Blair medium**
  - BioFire GI Panel: 23 targets – 14 bacteria, 4 protozoa, 5 viruses
  - **PCR detection for Giardia lamblia, Cryptosporidium, Cyclospora, E. histolytica, T. vaginalis, T. cruzi. T. gondii, etc.**
  - Microsporidia PCR performed at CDC and reference labs
- **Serology** (immunodiagnostic testing)
  - Serum: tests for **IgG and IgM antibodies to Toxoplasma gondii** is primary means of diagnosis
  - Serology for helminth infections: difficult to confirm serologically but usually have increased IgE + Eosinophils – heterogeneous mixtures of Antigens with cross-reacting Antibodies
  - In parallel with low platelet count and high eosinophils in Summer months from CBC

The FilmArray Pouch (Nested PCR)



Reagent Storage

Chemical Circuit Board

Sample extraction & purification

1st stage multiplex PCR

2nd stage PCR

Stool Preparation Video

https://www.youtube.com/watch?v=OlcRTMW9YZY

Testing of Fecal Specimens Preserved in Formalin and PVA:
(*indicates special test)